Infection with *Anaplasma phagocytophilum* in a young dog: a case report

O. Melter[1,3], I. Stehlik[2], H. Kinska[1], I. Volfova[1], V. Ticha[1], D. Hulinska[3]

[1]Labvet, Clinical Veterinary Laboratory, Prague, Czech Republic
[2]Veterinary Clinic, Prague, Czech Republic
[3]National Institute of Public Health, Prague, Czech Republic

ABSTRACT: An 11-months-old male Golden Retriever occasionally found to have *Ixodes ricinus* ticks attached to the skin developed the acute onset of fever, lameness and inappetence followed by rapidly progressive depression, ataxia and reluctance to move. Inclusions (morulae) were observed in granulocytes. The blood analysis revealed severe thrombocytopoenia, lymphopoenia, eosinopoenia, elevation of alkaline phosphatase and hypercholesterolaemia, mostly suggestive of an *Anaplasma phagocytophilum* infection. The amplification of a DNA sequence specific for *Anaplasma phagocytophilum* and detection of specific antibodies supported the diagnosis. *Borrelia burgdorferi,* another tick-borne pathogen, or specific antiborrelial IgG antibodies were not detected. The dog was treated with oral doxycycline for 14 days: clinical symptoms resolved within six days.

Keywords: anaplasmosis; infection; dog

A. phagocytophilum is an obligate intracellular pathogen known to cause granulocytic infections in both animals and humans (Dumler et al., 2001; Cohn, 2003). The most common vector of *A. phagocytophilum* in Europe is the tick *Ixodes ricinus*. Pet animals are not a source of infection for humans but could serve as a sentinel (Bjoersdorff, 2002).

Canine granulocytic anaplasmosis has been documented sporadically worldwide, with prevalence varying widely among countries (Engvall et al.; 1996; Pusterla et al., 1998; Liddell et al., 2003; Bexfield et al., 2005; Shaw et al., 2005; Liebisch et al., 2006). The detection of morulae in granulocytes does not identify the agent to the species level and further testing is needed (Cohn, 2003; Sirigireddy and Ganta, 2005). Lester et al. (2005), Kirtz et al. (2000, 2005) and Pusterla et al. (1997) reported 5–37% of infected granulocytes in dogs with anaplasmosis. The culture detection of the agent is still not a routine method yet and serologic testing alone is not conclusive for diagnosis. The diagnosis of *A. phagocytophilum* infection is mostly achieved by an indirect immunofluorescence assay and by amplified DNA specific to the 16S rRNA gene of the agent (Kirtz et al., 2005). Although two pet dogs with granulocytic morulae in their venous blood were reported in the Czech Republic (Huml et al., 1996), the causative species was identified only in game animals and humans by Hulinska et al. (2002, 2004).

Clinical symptoms of the infection with *A. phagocytophilum* in dogs are not specific but usually include fever, lethargy, thrombocytopoenia, depression and anorexia (Engvall et al., 1996). In experimentally infected dogs only mild symptoms were described (Cohn, 2003).

Dogs might be coinfected with other tick-borne species such as *Ehrlichia* spp. or *Borrelia* spp. and the disease manifests itself primarily as acute or subacute arthritis in contrast to the infection in humans (Straubinger, personal communication). Antiborrelial antibodies can first be detected by ELISA in the serum of dogs between 4 and 6 weeks after infection, with their titre peaking in 90 to 120 days and remaining constant for at least 22 months after exposure (Straubinger, personal communication). Although PCR is sensitive and specific, negative results do not exclude the infec-

tion with *B. burgdorferi* (Cohn, 2003). Twenty-five percent of dogs healthy or suspected of borrelial or anaplasmal infection (n = 731) had significant titres for both infections. The coinfection with *A. phagocytophilum* and *B. burgdorferi* is more likely to induce illness in the dogs as compared to the infection with either organism alone (Beall et al., 2006).

Infections with other tick-borne agents – *Babesia* spp. and tick-borne encephalitis (TBE) virus – with typical clinical symptoms are also reported sporadically in dogs in the Czech Republic (Klimes et al., 2001).

We present a case report of a young dog that fully recovered after developing febrile illness, lameness, depression and ataxia as the acute onset of suspected tick-borne disease.

Case history

An 11-months-old pet male Golden Retriever found to have several *Ixodes ricinus* adult female ticks attached to the skin between March and June developed acute signs of infection at the beginning of June. On admission to the veterinary clinic (Day 0), the dog showed the severe acute onset of pyrexia (39.6°C), lethargy, inappetence and ataxia. It was not able to either stand or walk. The general clinical examination including the inspection and palpation of leg bones and joints did not reveal any additional physical abnormalities.

The baseline biochemistry screening (Spotchem, SP-4430, Arkray) showed hypercholesterolaemia and elevation of alkaline phosphatase level (Table 1). The remaining parameters tested (urea, creatinine, ALT, AST, GMT) were within the reference ranges. Intravenous fluid therapy with saline solution (60 ml/kg/24 h) was immediately initiated. Intravenous metamizol (25 mg/kg, Vetalgin inj., Intervet) and subcutaneous amoxicillin/clavulanate (12.5 mg/kg, Synulox, RTU, Pfizer) were given to control fever, pain and suspected infection.

On Day 2, the fever (40.5°C) and depression increased. Intravenous fluid therapy with saline solution was given again (80 ml/kg/24 h). Haematological (AL Cell Counter 871, AL Systeme) and biochemical (Vitalab Selectra, Merck; EasyLyte Plus, Medica) analyses revealed some values outside the reference ranges (Table 1). Severe thrombocytopoenia, lymphopoenia and elevated erythrocyte sedimentation rate (+83/h, adjusted for hematocrit +77/h, refer-

Table 1. Selected haematological and biochemical parameters of blood samples from the studied dog infected with *A. phagocytophilum*

Parameter	At baseline	Day 2	Week 3	Month 5	Reference range**
Thrombocyte (count)	ND	29*	287	299	200–500 × 10^9 /l
Leukocyte (count)	ND	10.1	10.6	12.3	6–17 × 10^9 /l
Band neutrophil (count)	ND	0.05*/505*	0	0	rel. 0–0.03/abs. 0–300 mm^3
Segmented neutrophil (count)	ND	0.87*/8 787	0.57*/6 042	0.62/7626	rel. 0.60–0.77/abs. 3 000–11 500 mm^3
Lymphocyte (count)	ND	0.03*/303*	0.30/3 180	0.34/4182	rel. 0.12–0.30/abs. 1 000–4 800 mm^3
Monocyte (count)	ND	0.05/505	0.06/636	0.02*/246	rel. 0.03–0.10/abs. 150–1 350 mm^3
Eosinophil (count)	ND	0*	0.07/742	0.02/246	rel. 0.02–0.10/abs. 100–1 250 mm^3
Phosphorus	ND	1.33	ND	1.62*	up to 1.50 mmol/l
Calcium	ND	2.82*	ND	2.70	up to 2.77 mmol/l
Cholesterol	11.0*	10.51*	ND	10.91*	2.74–7.65 mmol/l
Total protein	ND	64.0	ND	68.6	58–76 g/l
Albumin	ND	36.75	ND	39.09*	23–36 g/l
ALP	4.29	5.04*	ND	2.30	up to 2.60 μkat/l
Creatinine kinase	ND	4.25*	ND	1.16	up to 2.50 μkat/l

ND = not determined; *the value outside the reference range, **based on Bush (1996)

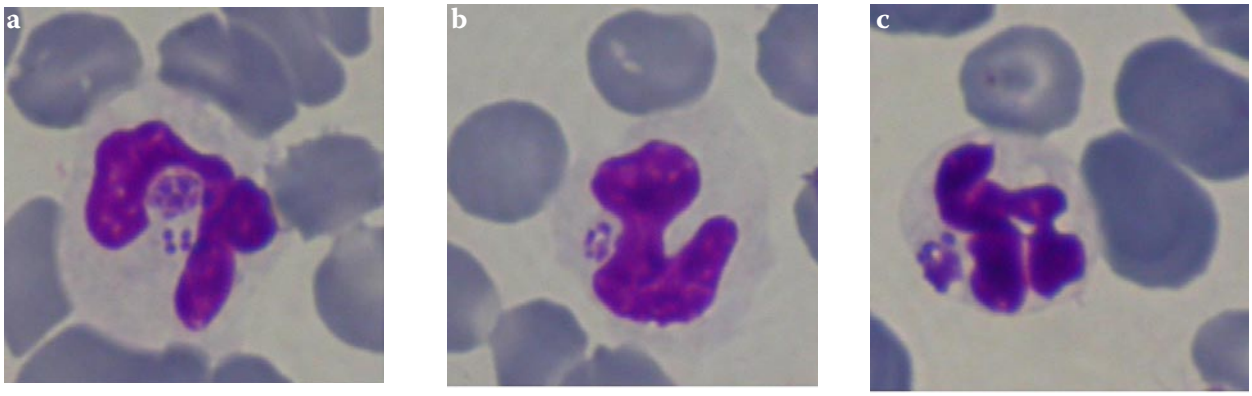

Figure 1 a–c. Blood smears from the acute onset of the illness. *Anaplasma phagocytophilum* morulae are evident within the dog granulocytes. Giemsa-Romanowski staining (magnification 1 000×)

ence range up to 10/1/h) were observed. Eosinophils could not be detected and the total number of leukocytes was within the reference range (Table 1). The other haematological parameters, i.e. haemoglobin, hematocrit and calculated characteristics, were within reference ranges (MCV 72.1 fl; MHC 14.5 fmol and MCHC 32.7g/dl). Giemsa-stained blood smears revealed the presence of isolated bacteria or inclusions (morulae) (Figure 1) in 36% of three hundred neutrophils.

Hypercholesterolaemia and elevation of creatinine kinase and alkaline phosphatase were observed (Table 1). Amylase activity was reduced (7.10 μkat/l, reference range 10–30 μkat/l). The other biochemical parameters tested (urea, creatinine, phosphorus, calcium, sodium, potassium, chloride, glucose, total protein, albumin, bilirubin, ALT, AST, GMT, and lipase) were in reference ranges. PCR analyses of *A. phagocytophilum* and *B. burgdorferi* sensu lato were performed as described by Jackson et al. (2002) and Marconi and Garon (1992), respectively. Template DNA was isolated from EDTA-collected blood specimens of the infected animal and five clinically healthy dogs as negative controls by a QiaAmpTissue kit (Qiagene). DNAs from the strain EHR 02 of *A. phagocytophilum* (Hulinska et al., 2004) and strain M192 of *B. garinii* (Hulinska et al., 1999) were used as positive controls. The 293 bp amplicon specific to *A. phagocytophilum* was detected only in the infected dog and the positive control (Figure 2) but it was not found in the healthy dogs. No amplicon specific to *B. burgdorferi* was detected in the blood specimens from either the infected animal or the five clinically healthy dogs. The ELISA test using whole cell *B. afzelii* antigen (Test-Line, Brno, Czech Republic) detected IgM to *B. burgdorferi* (index of positivity 1.40; positive result higher than 1.15) and IgG negativity in the blood of the infected dog. Since *A. phagocytophilum* and borrelial infection was suspected, the antibiotic was converted to 10 mg/kg doxycycline (Ronaxan, Merial) given orally twice daily for 14 days.

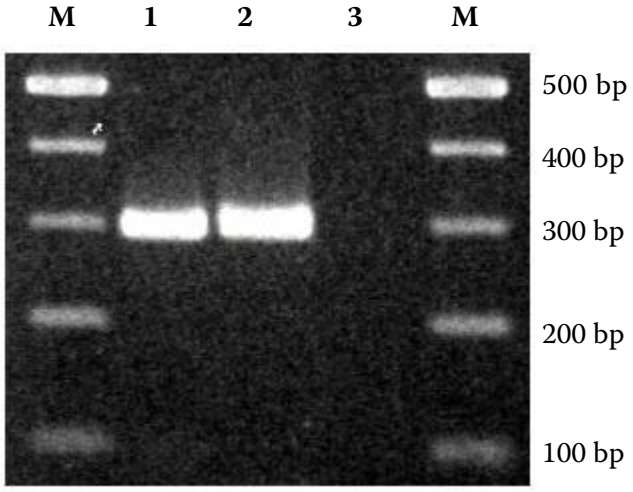

Figure 2. Agarose gel electrophoresis of 293 bp amplicons obtained with primers EHR521 and EHR790 from the infected dog's blood (1) and DNA extracted from *A. phagocytophilum* control strain EHR 02 (2). DNA extracted from five healthy dogs not included in the figure and PCR mixture (3) were used as negative controls analyzed with PCR. Lanes M show size standards (100 bp DNA ladder)

On the following day the temperature decreased to 39.0°C. Intravenous glucose in a 5% solution (20 ml/kg/24 h) and fluid therapy with vitamins (Duphalyte, Fort Dodge, 5 ml/kg/24 h) were administered.

On Day 5, the temperature further decreased to 38.5°C and the dog showed only slight lethargy. The clinical examination on Day 6 indicated full clinical recovery, with the temperature falling to 38.2°C. The therapy with doxycycline was continued for another 14 days.

Three weeks after the first examination all haematological parameters were within reference ranges. The recovered dog was followed up at monthly intervals.

Five months later, the laboratory analysis confirmed haematological and biochemical parameters to be in reference ranges, with the only exception of mild hypercholesterolaemia (Table 1). Neither anaplasmal nor borrelial DNA was detected in the convalescent venous blood five months after the acute onset of infection. ELISA of IgM and IgG specific to *B. burgdorferi* gave the same result as the baseline tests, i.e. the dog was IgM positive and IgG negative again. Specific IgG antibodies to *A. phagocytophilum* were detected in the convalescent serum by the indirect immunofluorescence assay (IFA) at a titre of 1:320 five months after the entry examination.

DISCUSSION

Canine granulocytic anaplasmosis usually presents as an acute febrile systemic illness (Stiles, 2000). Specific diagnostic tests include visualisation of specific morulae, anti-*Anaplasma* IgM and IgG antibody detection and PCR analysis, which is most reliable for early diagnosis (Engvall et al., 1996; Bjoersdorff, 2002).

The dog presented the most typical systemic clinical symptoms, i.e. fever, ataxia, depression, lameness, and laboratory results, i.e. – thrombocytopoenia, eosinopoenia, lymphopoenia and occurrence of band neutrophils (Bjoersdorf, 2002), suggestive of acute granulocytic anaplasmosis. However, other reported symptoms such as lymphadenopathy, splenomegaly and hepatomegaly (Neer, 1998) were not detectable by palpation.

The inspection and palpation of leg bones, muscles and joints did not reveal any physical abnormalities in the limbs. The reactivity to pain during palpation indicated that the nervous system was not involved. Even if no special neurological and musculoskeletal examinations were performed, the reluctance to move, typically reported in acutely infected animals (Engvall et al., 1996), seemed to be due to deep depression known to result from stress as a consequence of homeostasis imbalance due to inflammation.

Inclusions were observed in 36% of neutrophils: this proportion was close to the highest reported figure, i.e. 37% (Kirtz et al., 2000). The persistence of the infection was screened microscopically and by the amplification of specific *A. phagocytophilum* DNA in two consecutive blood samples collected three weeks and five months after the acute attack with negative results. However, Egenvall et al. (2000) detected *A. phagocytophilum* infection in three dogs for six months but caution would be advised in comparing their results obtained in the experimentally infected and immunosuppressed dogs with ours found in the naturally infected animal.

The amplification of the specific fragment of *A. phagocytophilum* and not of that of *Borrelia* spp. confirmed the infection with *A. phagocytophilum*. The positivity to antiborrelial IgM antibodies indicated either an exposure to *Borrelia* spp. or cross-reactivity with anaplasmal or other gram-negative bacterial surface antigens. The aetiological role of *Borrelia burgdorferi* in the case presented is disputable as no DNA and specific IgG antibodies were detected on admission and in convalescent specimens, while ELISA antibodies to whole cell or recombinant *Borrelia burgdorferi* antigens were reported to remain constant for nearly two years (Straubinger, personal communication).

The elevation of hepatic transaminase activity is presented in the literature (Bjoersdorff, 2002) rather than increased alkaline phosphatase (ALP), creatinine kinase (CK) and cholesterolaemia as described in our case history. The precise mechanisms for the elevations remain unknown. Elevated ALP and CK could probably be connected with septicaemia because of the effects of large numbers of multiplying bacteria and endotoxaemia. Bacterial endotoxins released in the blood could evoke liver and cardiac cell damage (Bush, 1996). The reasons for stable elevated cholesterol levels remain unclear. Increased lipolysis at the time of acute stress or starvation could be omitted because the level persisted five months after the successful therapy. An idiopathically increased serum choles-

terol level in the dog could be one reason explaining the phenomena (Bush, 1996).

The therapy with doxycycline proved as effective as found by others (Stiles, 2000; Cohn, 2003). The temperature started to decrease on day 2 and all clinical symptoms resolved on day 6 after the initiation of the therapy. At 3-week and 5-month follow-ups no clinical or laboratory changes except for elevated cholesterol were observed.

It is not likely that the dog was coinfected with another tick-borne agent. *Babesia* spp. was not seen microscopically in red blood cells and most of the typical clinical symptoms of babesios (anaemia, icterus, haemoglobinuria, dyspnoea and a low body condition index) did not appear in the dog (Taboada, 1998). The coinfection with TBE virus was possible but unlikely as the primary TBE symptoms typical of TBE infection (shyness and unwillingness, periods of excitement and irregular movements and high fever in the course of the disease) (Svoboda et al., 2001) were not observed and the dog responded quickly (within 24 hours) to the specific antibiotic therapy suggesting bacterial infection.

All findings summarized above, particularly the presence of specific morulae and DNA in neutrophils, negative laboratory or clinical results for other tick-borne agents, thrombocytopoenia and the fact that a high titre of specific IgG antibodies was detected in the convalescent serum suggest that *A. phagocytophilum* was the single causative agent of the acute infection. Other authors also reported the detection of specific IgG antibodies by IFA from 2 weeks up to more than six months after infection in dogs suffering from anaplasmosis (Kirtz et al., 2000, 2005; Poitout et al., 2005). To the author's knowledge this is the first confirmed case of canine anaplasmosis caused by *A. phagocytophilum* to be reported in the Czech Republic, where the infection was already suspected (Huml et al., 1996).

Acknowledgements

The authors thank Kamil Sedlak, DVM, PhD, from the State Veterinary Institute in Prague for measuring anti-borrelial antibodies and for the confirmation of anti-anaplasmal antibodies. Thanks are also due to Eva Kodytkova from the National Institute of Public Health in Prague for reviewing the manuscript.

REFERENCES

Beall M., Chandrashekar R., Eberts M. (2006): *Borrelia burgdorferi* and *Anaplasma phagocytophilum*: potential implications of co-infection on clinical presentation in the dog. Journal of Veterinary Internal Medicine, 20, 713–714.

Bexfield N.H., Villiers E.J., Herrtage M.E. (2005): Immune-mediated haemolytic anaemia and thrombocytopenia associated with *Anaplasma phagocytophilum* in a dog. Journal of Small Animal Practice, 46, 543–548.

Bjoersdorff A. (2002): Canine granulocytic ehrlichiosis due to *Anaplasma phagocytophila*. In: Beugnet F. (ed.): Guide to Major Vector-borne Diseases of Pets. Merial, France. 123–127.

Bush B.M. (1996): Interpretation of Laboratory Results for Small Animal Clinicians. Blackwell Science, New York. 273–276.

Cohn L.A. (2003): Ehrlichiosis and related infections. Veterinary Clinics: Small Animal Practice, 33, 863–884.

Dumler J.S., Barbet A.F., Bekker C.P., Dasch G.A., Palmer G.H., Ray S.C., Rikihisa Y., Rurangirwa F.R. (2001): Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. Journal of Systematic and Evolutionary Microbiology, 51, 2145–2165.

Egenvall A., Lilliehook I., Bjoersdorff A., Engvall E.O., Karlstam E., Artursson K., Heldtander M., Gunnarsson A. (2000): Detection of granulocytic *Ehrlichia* species DNA by PCR in persistently infected dogs. Veterinary Record, 146, 186–190.

Engvall E.O., Petterson B., Person M., Artusson K., Johansson K.E. (1996): A 16S rRNA-based PCR assay for detection and identification of granulocytic *Ehrlichia* species in dogs, horses, and cattle. Journal of Clinical Microbiology, 34, 2170–2174.

Hulinska D., Votypka J., Valesova M. (1999): Persistence of *Borrelia garinii* and *Borrelia afzelii* in patients with Lyme arthritis. Zentralblatt fur Bakteriologie, 289, 301–318.

Hulinska D., Votypka J., Plch J., Vlcek E., Valesova M., Bojar M., Hulinsky V., Smetana K. (2002): Molecular and microscopical evidence of *Ehrlichia* spp. and *Borrelia burgdorferi* sensu lato in patients, animals and ticks in the Czech Republic. New Microbiologica, 25, 437–448.

Hulinska D., Langrova K., Pejcoch M., Pavlasek I. (2004): Detection of *Anaplasma phagocytophila* in animals by real-time polymerase chain reaction. Acta Pathologica, Microbiologica et Immunologica Scandinavica, 112, 239–247.

Huml O., Cada F., Bohm, K., Velebny H. (1996): Granulocytic ehrlichiosis in a dog (in Czech). Veterinarstvi, 11, 464–466.

Jackson C.A., Lovrich S.D., Agger W.A., Callister S.M. (2002): Reassessment of Midwestern lyme disease focus for *Borrelia burgdorferi* and the human granulocytic ehrlichiosis agent. Journal of Clinical Microbiology, 40, 2070–2073.

Kirtz G., Leidinger E., Moser V. (2000): Canine granulocytare Ehrlichiose (CGE) bei einem Hund in Osterreich. Wiener Tierazliche Monatsschrift, 87, 241–246.

Kirtz G., Meli M., Leidinger E., Ludwig P., Thum D., Czettel B., Kolbl S, Lutz H. (2005): *Anaplasma phagocytophilum* infection in a dog: identifying the causative agent using PCR. Journal of Small Animal Practice, 46, 300–303.

Klimes J., Juricova Z., Literak I., Schanilec P., Trachta e Silva E. (2001): Prevalence of antibodies to tick-borne encephalitis and West Nile flaviviruses and the clinical signs of tick-borne encephalitis in dogs in the Czech Republic. Veterinary Record, 148, 17–20.

Lester S.J., Breitschwerdt E.B., Collis C.D., Hegarty B.C. (2005): *Anaplasma phagocytophilum* infection (granulocytic anaplasmosis) in a dog from Vancouver Island. Canadian Veterinary Journal, 46, 825–827.

Liddell A., Stockham S.L., Scott M.A., Sumer J.W., Paddock C.D., Gaudrealt-Keener M., Arens M.Q., Storch G.A. (2003): Predominance of *Ehrlichia ewingii* in Missouri dog. Journal of Clinical Microbiology, 41, 4617–4622.

Liebisch G., Thiet W., Liebisch A. (2006): Die canine monozytare (CME) und die canine granulozytäre Ehrlichiose (CGE), zwei durch Zecken ubertragene Infektionskrankheiten bei Hunden in Deutschland. Der Praktische Tierarzt, 87, 342–353.

Marconi R.T., Garon C.F. (1992): Development of polymerase chain reaction primer sets for diagnosis of Lyme disease and for species-specific identification of Lyme disease isolates by 16S rRNA signature nucleotide analysis. Journal of Clinical Microbiology, 30, 2830–2834. Erratum in: Journal of Clinical Microbiology, 1993, 31, 1026.

Neer T.M. (1998): Canine monocytic and granulocytic ehrlichiosis. In: Greene C.E. (ed.): Infectious Diseases of the Dog and Cat. 2nd ed. W.B. Saunders Co., Philadelphia. 139–147.

Poitout F.M., Shinozaki J.K., Stockwell P.J., Holland C.J., Shukla S.K. (2005): Genetic variants of *Anaplasma phagocytophilum* infecting dogs in Western Washington State. Journal of Clinical Microbiology, 43, 796–801.

Pusterla N., Huder J., Wolfensberger C., Litschi B., Parvis A., Lutz H.J. (1997): Granulocytic ehrlichiosis in two dogs in Switzerland. Journal of Clinical Microbiology, 35, 2307–2309.

Pusterla N., Pusterla J.B., Deplazes P., Wolfensberger C., Miller W., Horauf A., Reusch C., Lutz H. (1998): Seroprevalence of *Ehrlichia canis* and of granulocytic ehrlichia infection in dogs in Switzerland. Journal of Clinical Microbiology, 36, 3460–3462.

Shaw S.E., Binns S.H., Birtles R.J, Day M.J, Smithson R., Kenny M.J. (2005): Molecular evidence of tick-transmitted infections in dogs and cats in the United Kingdom. Veterinary Record, 157, 645–648.

Sirigireddy K.R., Ganta R.R. (2005): Multiplex detection of *Ehrlichia* and *Anaplasma* species pathogens in peripheral blood by real-time reverse transcriptase-polymerase chain reaction. Journal of Molecular Diagnostics, 7, 308–316.

Stiles J. (2000): Canine rickettsial infections. Veterinary Clinics of North America: Small Animal Practice, 30, 1135–1149.

Svoboda M., Pospisil Z., Svobodova V., Klimes J., Knotek Z., Smola J., Toman M., Matouch O., Literak I., Cizek A., Treml F., Celer V., Zendulkova D., Lany P., Doubek J., Schanilec P., Vernerova E., Halouzka R., Kohout P., Srenk P., Barton J., Hera A. (2001): Infectious and parasitic diseases. In: Svoboda M., Senior D.F., Doubek J., Klimes J. (eds.): Disease of the Dog and Cat (in Czech). Noviko a.s., Brno. 1840–1841.

Taboada, J. (1998): Babesiosis. In: Greene C.E. (ed.): Infectious Diseases of the Dog and Cat. W.B. Saunders Company, Philadelphia. 473–481.

Received: 2006–04–27
Accepted after corrections: 2007–04–11

Corresponding Author:

MVDr. Oto Melter, PhD., National Institute of Public Health, Srobarova 48, 100 42 Prague, Czech Republic
Tel. +420 267 082 255, e-mail: melter@szu.cz